Diabeto Protective Study of Various Extracts of *Argyrea Speciosa*, Sweet Root Extracts in Experimental Animals.

Pandurang M. Gaikwad[1], S. Vidyadhara[2] and Vikram V. Nimbalkar[1]

[1]PDVVPF's College of Pharmacy, Vilad Ghat, Ahmednagar, MS.
[2]Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur, AP.
*For correspondence – pmgaikwad09@yahoo.co.in, rajevikram@rediffmail.com

Summary

The various sutras in ayurveda illustrates that a person undergoing rejuvenation therapy attains longevity, memory, intellect, freedom from diseases, youth, excellence of luster, true sense-organ and respect, brilliance and vak-siddhi. This rejuvenation therapy is known as Rasayana therapy. The materials used in the therapy are termed as Rasayana. These Rasayana may be inducers of enzymes and hormones, which the body needs for adaptations and survival during normal health, stress and during the diseases also. Diabetes mellitus is a complex syndrome involving severe insulin dysfunction in conjunction with gross abnormalities in glucose homeostasis and lipid metabolism. The plant *Argyrea speciosa*, Sweet, is not explored scientifically for its medicinal values or for any of its use except few studies like antibacterial activity. There is a need for supplementation of exogenous antioxidants to protect the body from the onslaught of free radical induced damage, in such conditions. Hence our aim was to explore the dibetoprotective effect *Argyrea speciosa*, sweet root extracts on experimentally induced diabetes in animals. The roots were shade dried and powdered. The roots were defatted with petroleum ether (60-80º) and further extracted with distilled water (ASW), methanol (ASM) and under Soxhelet extraction and methanolic fraction of aqueous extract (ASMW) was prepared. The aqueous, methanolic and methanolic fraction of aqueous extract restored the blood glucose levels and along with restoration of serum triglycerides, cholesterol and lipoproteins which was altered on administration of streptozotocine. The results indicated potent diabeto-protective activity which may be attributed to ability of extracts to improve either the insulin reaction from B-cells or to because the carbohydrate absorption and it might be possible that these extracts might be counteracting the lipogenic which may have resulted in restoration of triglycerides and cholesterols.

Key words: Diabeto protective, *Argyrea speciosa*, streptozotocine

Introduction

The various sutras in ayurveda (1) illustrates that a person undergoing rejuvenation therapy attains longevity, memory, intellect, freedom from diseases, youth, excellence of luster, true sense-organ and respect, brilliance and vak-siddhi. This rejuvenation therapy is known as Rasayana therapy. The materials used in the therapy are termed as Rasayana. These Rasayana may be inducers of enzymes and hormones, which the body needs for adaptations and survival during normal health, stress and during the diseases also. Diabetes mellitus is a complex syndrome involving severe insulin dysfunction in conjuction with gross abnormalities in glucose homeostasis and lipid metabolism. The disease is generally broken down into two major subgroups (2). One group is insulin-dependent termed as type-I and the second is non-insulin dependent type-II. Much of the evidence concerning a role for oxidation in the induction of diabetes mellitus comes from the study of two drugs which induce diabetes in experimental animals; alloxan and streptozotocin (STZ) (3). The plant *Argyrea speciosa*, Sweet, is not explored scientifically for its medicinal values or for any of its use except few studies like antibacterial activity. There is a need for supplementation of exogenous antioxidants to protect the body from the onslaught of free radical induced damage, in such conditions. Hence our aim was to explore the dibetoprotective effect *Argyrea speciosa*, sweet root extracts on experimentally induced diabetes in animals.

Material and methods

Collection of plant material: The roots of A. speciosa, sweet were collected from the botanical garden of Ayurveda Mahavidyalaya, Panchavati, Nashik and the roots of the plant were authenticated by Dr. D. R. Mahajan, Head, Dept. of Botany, K.T.H.M. Science College, Gangapur Road, Nashik.

Preparation of *Argyrea speciosa*, Sweet Extracts: The roots were shade dried and powdered. The roots were defatted with petroleum ether (60-80º) and further extracted with distilled water (ASW), methanol (ASM) and under Soxhelet extraction and methanolic fraction of aqueous extract (ASMW) was prepared.

Diabeto-protective studies: Adult albino rats (Wistar strain) of either sex weighing 200-250gm were used in the entire study. Each pair of animal was housed in a spacious polypropylene cage containing wood shavings as nesting material, which was maintained at 23ºC ± 3°C in a well-ventilated animal house under natural photoperiod condition. The study protocol for the current study was prepared as per CPCSEA. All the study protocols were approved by Institutional Animal Ethical Committee of PDVVF's College of Pharmacy, Vilad Ghat, Ahmednagar. All the experiments were started only after the approval of IAEC and completion report was also submitted to the IAEC after the completion of experiments.

Methodology: Induction of Diabetes

Diabetes was produced by single yail vein injection of streptozotocin (45 mg/kg) dissolved in citrate buffer (0.2 M, pH 4.2). Control animals were administered normal saline. After 72 hours blood was collected from tail, & diabetes was confirmed when random blood glucose level increased more that 3 times its normal values.

Animals with fasting blood glucose level more than 300 mg/dl were considered diabetic and used for further studies. After confirming diabetes, the animals were administered drug orally for a period of 21 days. At the end of treatment schedule blood glucose, serum triglyceride, serum Total cholesterol, serum HDL cholesterol, serum LDL cholesterol levels were determined for dibeto-proctective activity.

Statistical Analysis: All observations were presented as Mean ± SEM. The data was analyzed by student's t-test for in-vitro studies and one-way ANOVA followed by Dunnett's test for in-vivo study. $p < 0.05$ is considered as significant and $p < 0.001$ as highly significant.

Grouping and treatment: The animals were divided into eight separate group's consisting of six animals each. Group 1 (Control): Animals were injected 0.2ml of normal saline and received water *ad libitum* along with normal diet. Group 2 (Streptozotocin): Diabetic control (STZ 45mg/kg., i.v.). Control diabetic group wherein Streptozotocin (45mg/kg., i.v.) was injected and the animals were given water *ad libitum* along with normal diet. Group 3 (ASW): Animals received aqueous extract ASW (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*. Group 4 (ASW + STZ): Animals received Streptozotocine (45mg/kg., i.v.) and aqueous extract ASW (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*. Group 5 (ASM): Animals received methanolic extract ASM (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*. Group 6 (ASM+STZ): Animals received Streptozotocine (45mg/kg., i.v.) and methanolic extract ASM (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*. Group 7 (ASWM): Animals received methanolic fraction of aqueous extract ASWM (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*. Group 8 (ASWM+STZ): Animals received Streptozotocine (45mg/kg., i.v.) and methanolic fraction of aqueous extract ASWM (1ml/kg of body weight, i.p. per day) along with normal diet and water *ad libitum*.

Results and discussion

The blood sugar levels (BSL) were increased significantly ($p<0.001$) in group 2 (STZ) as compared to group 1 (Control) (Table 1). STZ is a B-Cell cytotoxic agent. Administration of STZ cause damage to the B-Cells as a result of which there is a deficiency of insulin in the experimental animals causing significant increase in the blood glucose level (4). This effect of STZ not only confined to BSL but it also affects lipid levels in animals. These animals exhibit low endogenous production of insulin with high levels of circulating glucose. STZ treated rats are catabolic in nature and tissues exhibit insulin resistance despite increased in insulin binding. (5) STZ treated rats show hyperlipidaemia these abnormalities have been reversed by administration of Argyrea speciosa extracts during the experimental period (6).The BSL levels were decreased significantly ($P<0.001$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ). This may be because of ability of extracts to improve either the insulin reaction from B-cells or to because the carbohydrate absorption in experimental animals many herbal preparations extracts has shown the significant reduction in the BSLs in animals after exposure to STZ.

The serum triglyceride levels (TGLY) were increased significantly ($p<0.01$) in group 2 (STZ) as compared to group 1 (Control) (Table 2). The TGLY levels were decreased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ). The serum cholesterol levels (CHOL) were increased significantly ($p<0.01$) in group 2 (STZ) (Table 3) as compared to group 1 (Control). The CHOL levels were decreased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ). The serum high density lipoprotein cholesterol levels (HDL) were decreased significantly ($p<0.01$) in group 2 (STZ) (Table 4) as compared to group 1 (Control). The HDL levels were increased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ). The serum low density lipoprotein cholesterol levels (HDL) were increased significantly ($p<0.01$) in group 2 (STZ) (Table 5) as compared to group 1 (Control). The LDL levels were decreased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ). The levels total cholesterol, triglyceride, LDL- cholesterol was significantly increased in STZ treated animals and the levels of HDL- cholesterol were found to be significantly decreased in STZ treated animals. Earlier reports of (7) have showed prominent increase in serum and tissue lipid levels in rats treated with STZ. The increase of serum lipids by STZ was found to be very prominent. It was observed in our study that, the *Argyrea speciosa* extracts i.e. ASW; ASM & ASWM have hypolipiodmeic property. It might be possible that these extracts might be counteracted the lipogenic effect at sucrose alcohol & cholesterol diets (8). The extracts could only counter act the high LDL levels in STZ treated rats. The extracts produced conspicuous effects by antagonizing the elevated levels of serum lipids in animals. This might be because of either enhancing cholesterol metabolism via HDL-cholesterol which constitutes 50% of total cholesterol. It has also found that there was significant increase in HDL cholesterol levels in this present study. Decreased levels of HDL are always formed in phenotypes, who is frequently associated with visceral obesity, hyper insulinism and mild hypertension, a syndrome referred as syndrome 'X' or the insulin resistance syndrome such individuals constitute a group of unusually high risk for early cardiovascular disease (9)

Macrophages do not form cells with native LDL but oxidatively modified LDL (OLDL) is actively formed to the scavenger receptors on the macrophages which serve to clear toxic anomic macromolecules such as OLDL from the circulation (10). There is considerable direct and indirect evidence that oxidatively modified LDL component is demonstrated within the lesions on the line with the above STZ to produced prominent increase in lipid levels in experimental animals (11). The ASW, ASM & ASWM extracts produced a conspicuous effect by antagonizing the elevated levels of serum lipids in animals. It was the peculiar observation that the extracts ASW, ASM & ASWM are reducing the lipid levels in experimental animals suggesting that these extracts may enhance the cholesterol catabolism via HDL cholesterol which constitute about 50% of the total cholesterol. Hypolipidemic effects of ASW, ASM & ASWM extracts was observed to a maximum extent in lowering high density LDL cholesterol and increase in HDL cholesterol levels in experimental animals. All the extracts under evaluation increased the HDL cholesterol levels thus it may be useful in diseases like DM & CHD because of their inverse relationship.

Hypertriglycermia is also associated in metabolic consequences of hypercoagulability insulinemia, insulin resistance & glucose tolerance (12). Treatment with all the extracts has significantly reduced the levels at triglycerides in serum of animal exposed to STZ. This may prevent the progression of atherosclerosis and complications of hypertriglyceridemia (13).

Table 1:

Effects of administration of *Argyrea speciosa*, sweet extracts on blood sugar (Glucose) levels.

Group No	Groups	BSL (mg/dl)
1	Control	102.5 ± 6.8
2	STZ	384.6 ± 9.3[C]
3	ASW	101.2 ± 5.6
4	ASW+STZ	198.6 ± 7.8[C]
5	ASM	102.9 ± 4.8
6	ASM+STZ	165.6 ± 6.2[C]
7	ASWM	105.2 ± 3.6
8	ASWM+STZ	178.6 ± 7.2[C]

Table 2:

Effects of administration of *Argyrea speciosa*, sweet extracts on serum Triglycerides (TGLY).

Group No	Groups	TGLY (mg/dl)
1	Control	12.19 ± 0.61
2	STZ	34.79 ± 0.52[b]
3	ASW	12.01 ± 0.54
4	ASW+STZ	26.41 ± 0.35[a]
5	ASM	12.65 ± 0.33
6	ASM+STZ	24.25 ± 0.32[a]
7	ASWM	11.95 ± 0.33
8	ASWM+STZ	19.25 ± 0.32[a]

Table 3:

Effects of administration of *Argyrea speciosa*, sweet extracts on serum Cholesterol (CHOL).

Group No	Groups	CHOL (mg/dl)
1	Control	67.52 ± 0.97
2	STZ	88.42 ± 0.73[b]
3	ASW	68.12 ± 0.92
4	ASW+STZ	80.02 ± 0.62[a]
5	ASM	68.23 ± 0.84
6	ASM+STZ	76.89 ± 0.52[a]
7	ASWM	67.23 ± 0.84
8	ASWM+STZ	71.89 ± 0.52[a]

Table 4:

Effects of administration of *Argyrea speciosa*, sweet on serum High Density Lipoprotein Cholesterol (HDL).

Group No	Groups	HDL (mg/dl)
1	Control	33.6 ± 1.93
2	STZ	16.4 ± 1.62[b]
3	ASW	33.40 ± 1.81
4	ASW+STZ	25.60 ± 2.36[a]
5	ASM	32.90 ± 1.35
6	ASM+STZ	21.35 ± 2.52[a]
7	ASWM	32.80 ± 1.81
8	ASWM+STZ	20.81 ± 1.56[a]

Table 5:

Effects of administration of *Argyrea speciosa*, sweet extracts on serum Low Density Lipoprotein Cholesterol (LDL).

8	ASWM+STZ	38.2 ± 3.31[a]
Group No	Groups	LDL (mg/dl)
1	Control	35.3 ± 1.84
2	STZ	83.6 ± 2.35[b]
3	ASW	34.9 ± 2.61
4	ASW+STZ	56.3 ± 3.20[a]
5	ASM	35.5 ± 3.21
6	ASM+STZ	55.3 ± 2.32[a]
7	ASWM	35.2 ± 2.35

References

1) Sharma, N., Garg, V. (2009). Antidiabetic and antioxidant potential of ethanolic extract of Butea monosperma leaves in alloxan-induced diabetic mice. Indian *J Biochem Biophys*., 46(1), 99-105.
2) Xie, JT., Wang, CZ., Li, XL., Ni, M., Fishbein, A., Yuan, CS. (2009). Anti-diabetic effect of American ginseng may not be linked to antioxidant activity comparison between American Ginseng and Scutallaria baicalensis uning as ob/ob mice model. *Fitoterapia*., 80(5), 306-311.
3) Watala, C., Kazmierczak, P., Dobaczewski, M., Przygodzki, T., Bartus, M., Lomnicka, M., Slominska, EM., Durackova, Z., Chlopicki, S. (2009). Anti-diabetic effects of 1-methylnicotinamide (MNA) in streptozocin-induced diabetes in rats. *Pharmacol Rep*., 61(1), 86-98.
4) Song, MY., Kim, EK., Lee, HJ., Park, JW., Ryu, DG., Kwon, KB., Park, BH. (2009). Fructus Xanthii extract protects against cytokine-induced damage in pancreatic beta-cells through suppression of NF-kappaB activation. *Int J Mol Med*., 23(4), 547-53.
5) Meyerovithch J, Farfelz, Sack J, Schechter Y. (2002). Oral and – of vanadate normalize BSL in STZ-traeted rats. *J Biol-Chem., 202*, 6658.
6) Devore, EE., Stampfer, MJ., Breteler, MM., Rosner, B., Hee Kang, J., Okereke, O., Hu, FB., Grodstein, F. (2009). Dietary fat intake and cognitive decline in women with type 2 diabetes. *Diabetes Care*., 32(4), 635-640.
7) Jaiswal, D., Kumar, Rai P., Kumar, A., Mehta, S., Watal, G. (2009). Effect of Moringa oleifera Lam. Leaves aqueous extract therapy on hypoglycemic rats. *J Ethnophamacol*., 125 (3), 392-396.
8) Mortensen, LS., Hartvigsen, ML., Brader, LJ., Astrup, A., Schrezenmeir, J., Holst, JJ., Thomsen, C., Hermansen, K. (2009). Differential effects of protein quality on postprandial lipemia in response to a fat-rich meal in type2 diabets: comparison of whey, casein, gluten, and cod protein. *Am J Clin Nutr*., 90(1), 41-48.
9) Tie, L., Li, XJ., Wang, X., Channon, KM., Chen, AF. (2009). Endothelium-specific GTP cyclohydrolase I overexpression accelerates refractory wound healing by suppressing oxidative stress in diabetes. *Am J Physiol Endocrinol Metab*., 296(6), 1423-1429.
10) Matsuda, H., Kiyohara, S,, Sugimoto, S., Ando, S., Nakamura, S., Yoshikawa, M. (2009). Bioactive constituents from Chinese natural medicines. XXXIII. Inhibitors from the seeds of Psoralea corylifolia on production of nitric oxide in lipopolysaccharide-activated macrophages. *Biol Pharm. Bull*., 32(1), 147-149.
11) Luo, YD., Chen, J., Cao, J., Wen, XD., Li, P. (2009). Determination of sweroside in rat plasma and bile for oral bioavailability and heparobiliary excreation. *Chem Pharm.Bull*., 57 (1), 79-83.
12) Ginsberg, HN. (1994). Lipoprotein metabolism and its relationship to atherosclersosis med. *Cli North Am*., 78, 1-20.
13) Anstin, NA., Mokanson, JE. (1994). Epidemiology of trighycerides small dense low density lipoprotein metabolism. New York: H Greten, Springer-verlag, 119-124.